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Quantitative Determination of the Nutritional Status of Detrital Microbiota and the Grazing Fauna by Triglyceride Glycerol Analysis

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16. ABSTRACT

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QUANTITATIVE DETERMINATION OF THE NUTRITIONAL STATUS OF DETRITAL MICROBIOTA AND THE GRAZING FAUNA BY TRIGLYCERIDE GLYCEROL ANALYSIS

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Abstract: Endogenous lipid storage components are accumulated or utilized by both microorganisms and marine invertebrates, depending upon their nutritional status. Triglycerides are commonly the lipid endogenous storage materials utilized by fungi, marine vertebrates and many invertebrates, and can be quantitatively estimated by gas chromatographic assay of triacyl glycerol from lipid extraction. Deprivation from a food source can be detected by loss of triglyceride glycerol and estimated as the ratio of triglyceride glycerol to phospholipid measured as extractable lipid phosphate. In several estuarine animals, deprivation from food sources resulted in decline of both neutral lipid glycerol and triglyceride glycerol. Amphipods, feeding on estuarine detritus in the laboratory, showed the same nutritional state as those taken from detrital baskets in the field and were considerably better nourished than control amphipods deprived of food for a week.

INTRODUCTION

In attempting to describe interactions at the base of estuarine and marine benthic food chains, it is important to estimate quantitatively the effects of various perturbations on the biota. A quantitative estimation of the nutritional status would be a very useful index of the physiological condition of organisms in the system. The adenylate energy charge is a measure of the instantaneous physiological status of organisms from bacteria to mammals (Knowles, 1971), that can respond in seconds to various serious perturbations. The adenylate energy charge is readily measured from the adenosine nucleotides quantitatively recovered from the water phase of a lipid extraction (Davis & White, 1980). A much more sensitive measure of instantaneous physiological status is the detection of an increased rate of adenosine formation from the hydrolysis of adenosine monophosphate. The adenosine is formed in one of the homeostatic compensatory reactions by which the adenylate energy charge is maintained in the face of falling adenosine triphosphate formation (Davis & White, 1980).

A measure of the nutritional status which integrates the physiological status over a longer term can be assessed by following the rates of formation and catabolism of the endogenous storage materials. These endogenous storage materials are formed during periods of food surfeit and utilized during periods of deprivation. In the prokaryotes, the endogenous lipid polymer poly beta-hydroxybutyrate (PHB) is formed under conditions where carbon and energy sources are available, but the lack of some critical

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nutrient prevents microbial doubling (Dawes, 1976). PHB is readily measured during periods of unbalanced growth in environmental samples (Nickels *et al.*, 1979). In eukaryotes two types of endogenous storage materials predominate: the glucose polymers starch in plants and glycogen in animals and some bacteria, and the lipids, triglycerides and waxes in animals. Plants primarily form the polyglucose starches as endogenous storage materials. The carbohydrate endogenous storage materials are formed directly from the carbohydrate degradative pathways and their synthesis usually entails no problems in osmoregulation. However, the water of hydration of the glucose polymers and the relatively poor energy yield makes them bulky. The lipid endogenous storage materials of eukaryotes are primarily triglycerides in terrestrial organisms and mixtures of triglycerides and waxes in the aquatic world. Algae and diatoms form triglycerides primarily during periods of nutritional stress, such as nitrogen limitation (Fogg, 1975; Jorgensen, 1977). Fungi utilize triglycerides as endogenous storage materials (Weete, 1980). Vertebrates on land and sea rely exclusively on triglycerides as a long-term endogenous energy and carbon reserve (Pond, 1978). Glycogen furnishes the carbohydrate that regulates the utilization of the triglyceride fatty acids in vertebrates. Invertebrates utilize glycogen, triglycerides, and waxes as endogenous storage materials. Glycogen tends to be utilized by marine adult animals in which the limitations of space and streamlining are less critical to survival, such as hard shelled mollusks or echinoderms (Slobodkin, 1962), or in animals with a steady food source (Holland, 1978). Triglyceride is the primary endogenous storage material utilized for differentiation in the egg, for larval metamorphosis (especially during periods of starvation), and for molting (Holland, 1978). Waxes tend to be utilized in animals whose life cycles include long periods of starvation and where their lower specific gravity (0.86 vs. 0.92 for triglycerides) can provide buoyancy (Benson & Lee, 1975; Sargent *et al.*, 1976). In organisms containing both triglycerides and waxes, the triglycerides are utilized more readily than the waxes (Sargent *et al.*, 1976).

This study reports a method by which the triglyceride content of the microeukaryotes and their primary grazers can be measured. Triglycerides are heterogeneous, containing a wide range of fatty acids, none of which appears to be specific for triglycerides. Thus an assay for the constant glycerol portion of the molecule was developed. This study will show that a relatively rapid, sensitive, and quantitative gas chromatographic assay for triglyceride glycerol can be applied to define the nutritional status of the eukaryote microbiota and its grazers. An attempt has thus been made to compare triglyceride levels in fed and unfed populations from a wide variety of macro- and microorganisms, both from the field and from laboratory cultures, and to seek a consistent pattern that would indicate nutritional status.

Materials and Methods

COLLECTION OF ORGANISMS

Detrital microbiota

Teflon sheets 5 μm thick and 5 cm square were strung on nylon monofilament lines in groups of 50 in 2.5-cm mesh hardware cloth baskets 1 m deep in the estuary adjoining the Florida State University marine laboratory (29°54.0′N : 84°37.8′W). The strings of Teflon squares were suspended in the estuary for 3 wk in June, 1981, removed from the baskets, the macrobiota washed free, and transported in oxygenated sea water to the laboratory where they were suspended in roughly 20 times their volume of aerated sea water to which 0.002% (w/v) nutrient broth and 100 μM glucose were added. The Teflon squares were incubated for 3 days at ambient light. These were considered "fed". The "unfed" detrital microbiota was suspended in sterile sea water made from distilled water and Instant Ocean (Aquarium Systems, Inc., East Lake, Ohio) for 3 days at pH 7.95 without light. At the end of the experiment the Teflon surfaces with the attached detrital microbiota were lyophilized.

Estuarine animals

Grass shrimps *Palaemonetes pugio* (Holthius) were recovered from the baskets and frozen immediately in liquid nitrogen (fed) or incubated for 10 days in sea water filtered through a 0.45-μm filter (unfed). The shrimp were kept from cannibalism and coprophagy by isolation in separate bowls with water changes at least twice daily. Thirteen shrimp (average dry wt = 46.6 mg) were used for the fed experiments and 9 shrimp (average dry wt = 33.2 mg) for the unfed experiments.

Amphipods, (equal numbers of *Melita appendiculata* (Say) and *Lembos websteri* (Bate)) were recovered from the baskets and frozen immediately with liquid nitrogen (fed sample, 210 animals of average dry wt = 1.79 mg) or after suspension for 21 days in filter sterilized sea water (unfed sample, 180 animals, of average dry wt = 0.83 mg).

Sand dollars *Mellita quinquiesperforata* (Leske) were taken from the Bay Mouth bar sediments (29°54.5′N : 84°26.5′W) (White *et al.*, 1980) and quick frozen with liquid nitrogen (fed sample, 2 animals average dry wt = 34.7 g) or held in filtered sterilized sea water for 7 days (unfed sample, two animals average dry wt = 36.6 g).

The frozen animals were ground to powder in a mortar and pestle, divided into four portions, and extracted as described below.

Fungi

Wild type *Neurospora crassa* FGSC 988 79CA (the gift of Dr. R. M. DeBusk) was grown on Vogel's medium N (Vogel, 1964) supplemented with 2% (w/v) sucrose for 3 days. Fragments of the mycelial mat were transferred to media containing sucrose (fed sample) or media not containing sucrose for an additional 3 days at 25 °C (unfed).

The yeast *Saccharomyces cerevisiae* S7C (the gift of Dr. S. DeKloet) was grown on a medium containing 2% (w/v) glucose, 2% peptone, and 1% yeast extract made to pH 5.0 for 24 h. The culture was washed in sterile 100 μM sodium phosphate buffer to the same medium (fed sample) or the phosphate buffer (unfed sample) and harvested by centrifugation after 24 h. Samples were lyophilized prior to extraction.

BIOCHEMICAL METHODS

Materials

Nanograde or glass distilled solvents (Burdick and Jackson, Muskegon, Mich.) and freshly distilled chloroform were utilized for extraction and derivatization. Lipid standards and derivatizing reagents were purchased from Pierce Chemical Co., Rockford, Ill., Aldrich Chemical Co., Inc., Milwaukee, Wisc., and PCR Research Chemicals, Inc., Gainesville, Fl.

Lipid extraction

Lyophilized samples of fed and unfed animals, cultures, or detrital microbiota were extracted by the modified Bligh & Dyer (1959) single-phase chloroform-methanol

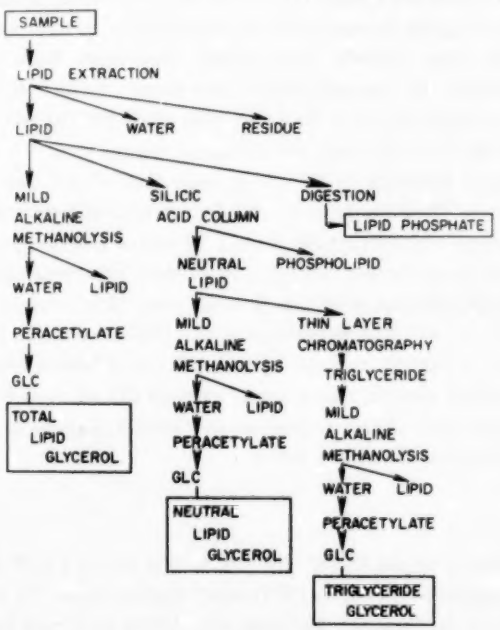

Fig. 1. Flow diagram of the analyses used in this study: GLC, gas-liquid chromatography.

method (White *et al.*, 1979), the phases separated by adding additional buffer and chloroform and the chloroform phase filtered through fluted folded Whatman 2V filter paper. A portion of the chloroform phase was removed for perchloric acid digestion and the colorimetric determination of the lipid phosphate (White *et al.*, 1979). The chloroform was removed in vacuo at 40 °C and the lipids stored under nitrogen at −10 °C. The analysis scheme utilized in this study is given in Fig. 1.

Column chromatography

A portion of the lipid was dissolved in chloroform and fractionated on silicic acid (Unisil, 100–200 mesh). Columns 0.54 × 29 cm were packed with 1 g silicic acid suspended in chloroform and the lipids added at < 0.4 mg dry wt lipid per g of silicic acid. The neutral lipids were recovered by elution with 10 ml of chloroform. The glycolipids and phospholipids can also be recovered quantitatively (King *et al.*, 1977).

Thin-layer chromatography

Whatman K6 silica gel thin layer plates of 250-μm thickness were spotted with the neutral lipid fraction and the triglycerides separated by ascending chromatography with a solvent of petroleum ether : diethyl ether : acetic acid, 80 : 20 : 1 (v/v). Outside bands spotted with tripalmitin or tristearin were included. At the end of the chromatography, the center portion (that contained the sample) of the plate was covered with Saran Wrap and the plate lightly sprayed with rhodamine 6G and the triglyceride band detected by its fluorescence in ultraviolet light. The silicic acid corresponding to the triglycerides at R_f 0.4 – 0.5 was scraped off and the triglycerides eluted with 10 ml of chloroform : methanol, 1 : 1, v/v, from a column made from the powder.

Mild alkaline methanolysis

The lipids were dissolved in 0.5 ml of methanol : toluene, 1 : 1 (v/v) to which an equal volume of freshly prepared 0.2 M methanolic KOH was added, mixed on a Vortex mixer, and incubated for 15 min at 40 °C in a test tube with a Teflon lined screw cap. The mixture was neutralized to pH 5.0 with equimolar 10% (v/v) acetic acid and partitioned by adding 2 ml each of chloroform and water, mixing for 5 min with the Vortex mixer, centrifuging, and recovering the water. The process was repeated. The chloroform was washed by adding a third 2-ml portion of water to wash down the sides of the test tube which was recovered without mixing and added to the water washes. The combined water washes were reduced to dryness in vacuo at 40 °C.

Peracetylation

The internal standard of 0.2 μmol of 1,9-nonanediol (Aldrich Chemical Co., Inc., Milwaukee, Wisc.) dissolved in methanol was added to Teflon lined screw cap test tubes containing the glycerol from the lipid samples and the solvent removed in a stream

of nitrogen at 30–40 °C. One ml of acetic anhydride : pyridine, 1 : 1 (v/v) was added, the solution mixed with a Vortex mixer, and heated to 60 °C for 2 h. The test tube was cooled and 2 ml of chloroform added. The solution was washed with an aqueous 20% (w/v) tartaric acid solution with Vortex mixing and centrifugation. The water was removed and the washing repeated twice more. The chloroform was removed in a stream of nitrogen and the sample dissolved in tetrahydrofuran for gas-liquid chromatography (GLC).

Gas-liquid chromatography

Samples of triacetylated glycerol were analyzed with a Varian 3700 gas chromatograph with a CDS 111 controller and a model 8000 autosampler. Data were fed into a programmable Hewlett-Packard 3502 laboratory data system. One μl of sample was injected onto a 25-m, 0.25-mm internal diameter glass capillary column coated with the polar cyanoalkylpolysiloxane liquid phase Silar 10C (Applied Science Co., Inc., State College, Penn.) operated in the splitless mode with 0.5-min venting time. The temperature program initiated at 60 °C and increased to 115 °C at 5°/min followed by a 2°/per min rise to 190 °C and a 12-min isothermal period. The helium carrier gas flow rate was 1.5 ml/min at 0.92 kg/cm^2. The injection port was at 220 °C and the detector at 250 °C. Under these conditions the response factor of triacetylglycerol to diacyl 1,9-nonanediol was 2.0.

Mass spectrometry

Capillary gas-chromatographic/mass-spectral fragmentography was performed with the Hewlett-Packard 5995A GC/MS using the same column and conditions as for the GLC. The mass spectrometer was autotuned with decafluorotriphenylphosphine with a 70 meV fragmentation energy. Spectra were recorded at a scan speed of 380 AMU/s (4 samples/0.1 AMU) with a 0.5-s delay between scans of 50 to 350 AMU. The spectrometer was operated in the peakfinder mode at an electron multiplier voltage of 1400 V. The threshold of detectability was set at 100 linear counts.

Statistical analysis

One-way analysis of variance for each parameter was performed using program 26-1705 with a Radio Shack TRS 80 computer. Significant differences between means at alpha = 0.05 are indicated by * in the tables.

Results

CHROMATOGRAPHY

Triacylglycerol is readily eluted from a 25-m Silar 10C coated glass capillary gas-chromatographic column (Fig. 2) with a calculated efficiency of n = 23400 theoretical

plates with n/m = 936 (m = 25 meters). The putative triacylglycerol gave a fragmentation pattern of m/e 103 (M – 73 – 43 + 1) = 100%; m/e 145 (M – 73) = 76.3%; m/e 116 (M – 59 – 43) = 45.2%; m/e 115 (M – 103) = 31.8%; m/e 86

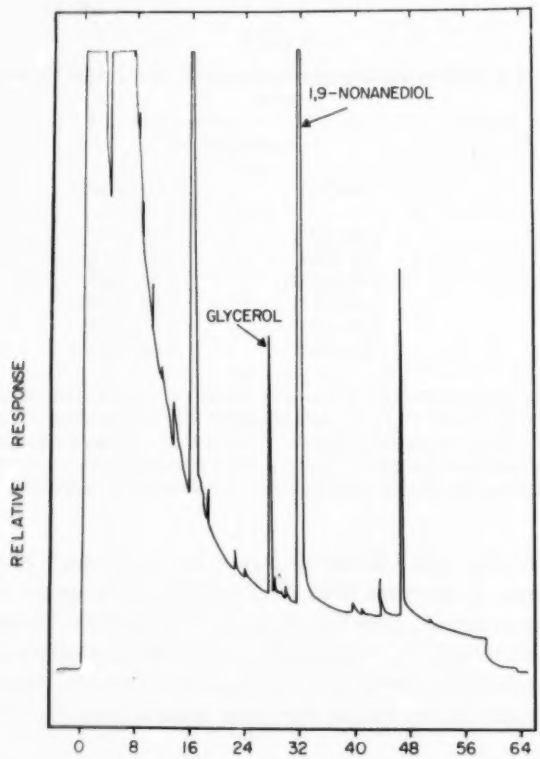

Fig. 2. Gas chromatographic tracing of the separation of triacylated glycerol (glycerol) and the internal standard diacylated 1,9-nonanediol as they eluted from a 25-m Silar 10C coated glass capillary column under the conditions described in the text.

(M – 73 – 59) = 19%; m/e 73 (M – 145) = 14.9% which was identical to authentic triacylglycerol.

SENSITIVITY

With the flame ionization detector operating at a sensiuivity of 4×10^{-12} A/mV, 0.01 nmol of triacylglycerol gave an integrator response twice the background.

RECOVERY

Authentic standards were put through the various portions of the procedures of Fig. 1 and the recoveries determined (Table I). Glycerol and tripalmitin can be quanti-

TABLE I

Recoveries of glycerol, tripalmitin and dimyristoyl phosphatidyl choline from the glycerol analysis procedure.

Procedure	μmol glycerol		% Recovered
	Added	Recovered	
Glycerol, hydrolysis[a]	2.00 (0.02)	1.68 (0.27)	84.1 (10.9)
Glycerol, volatility	2.00 (0.02)	1.77 (0.04)	88.7 (17)
Tripalmitin, hydrolysis	1.00 (0.015)	1.03 (0.09)	103 (9)
Tripalmitin, column	1.00 (0.02)	1.06 (0.09)	106 (8.8)
Tripalmitin, TLC	1.00 (0.02)	1.06 (0.15)	105 (15)
Dimyristoyl PC	2.00 (0.02)	0.012 (0.013)	0.3 (0.7)

[a] Data as $\bar{X}$ (SD) with glycerol after mild alkaline methanolysis, $n = 5$; with glycerol added to 5 ml water and water removed in vacuo at 40 °C, $n = 10$; with tripalmitin after mild alkaline methanolysis, $n = 5$; with tripalmitin eluted from silicic acid and mild alkaline methanolysis, $n = 4$; with tripalmitin eluted from silicic acid, purified by thin-layer chromatography, and mild alkaline methanolysis, $n = 4$; and with dimyristoyl phosphatidyl choline after mild alkaline methanolysis, $n = 7$; numbers in parentheses are SD.

tatively recovered after mild alkaline methanolysis in the water phase of the lipid extraction. Removal of the water from the extraction following the methanolysis by evaporation in vacuo did not cause loss of glycerol. Purification of tripalmitin from the lipid extract by silicic acid chromatography or by thin layer chromatography of the neutral lipid fraction did not result in a loss of glycerol from the tripalmitin. The mild alkaline methanolysis did not release significant glycerol from phosphatidyl choline.

HYDROLYSIS

To liberate the glycerol from triglycerides, the mild alkaline methanolysis (0.2 M methanolic KOH in toluene : methanol, 1 : 1, v/v) for 15 min at 40 °C was compared to mild acid methanolysis (methanol : chloroform : concentrated HCl, 10 : 1 : 1, v/v) for 2 h at 60 °C. The recovery of glycerol from tripalmitin with mild alkaline methanolysis was 97.4 (3.3)% ($\bar{X}$ (SD), $n = 5$) and from mild acid methanolysis was 93.1 (16.6)%. Because of the slightly better recovery from the alkaline methanolysis and the much greater stability of the deacylated glycerol phosphoryl esters, such as glycerol phosphoryl choline derived from phosphatidyl choline (White, 1968), the mild alkaline methanolysis was utilized.

EFFECT OF FEEDING ON THE TRIGLYCERIDE LEVEL

The glycerol derived from the total lipids, the neutral lipids, and the triglycerides decreased significantly in *Neurospora*, grass shrimp and amphipods (Table II). With

TABLE II

Effect of food deprivation on the glycerol derived from the total lipid, neutral lipid, and triglyceride as well as the total phospholipid of fungi, estuarine animals, and the detrital microbiota.

	Total lipid glycerol	Neutral lipid glycerol	Triglyceride glycerol	Lipid phosphate
Neurospora[a]				
Fed	31.9 (1.71)	29.4 (1.7)	16.6 (5.6)	9.6 (0.29)
Unfed	8.62 (0.4)*	6.32 (1.83)*	4.87 (0.71)*	30.3 (0.6)*
Yeast[a]				
Fed	5.18 (1.16)	2.37 (0.34)	3.81 (1.39)	15.4 (0.32)
Unfed	8.12 (1.55)*	4.1 (1.01)*	1.8 (0.26)*	22.2 (1.17)
Grass shrimp[b]				
Fed	–	80.8 (5.8)	23.6 (3.0)	2180 (270)
Unfed	47.7 (7.8)	20.7 (· 8)*	11.3 (1.2)*	1620 (330)*
Amphipods[b]				
Fed	24.8 (1.7)	14.9 (2.7)	12.6 (1.6)	142.1 (6.98)
Unfed	8.4 (5.4)*	3.5 (1.26)*	3.5 (0.96)*	40.1 (0.92)*
Sand dollars[b]				
Fed	–	–	590 (208)	6.81 (0.59)[c]
Unfed	–	–	985 (109)	5.91 (0.34)
Detrital microbiota[d]				
Fed	23.5 (3.2)	2.6 (0.8)	1.5 (0.5)	6.2 (2.1)
Unfed	37.6 (1.1)	1.0 (0.01)	1.9 (1.2)	21 (5)

[a] μmol glycerol or phosphate per g dry wt expressed as $\bar{X}$ (SD).
[b] nmol glycerol or phosphate per animal.
[c] mmol phosphate per animal.
[d] nmol glycerol or phosphate per cm^2.
* Significant differences (alpha = 0.05) between means by analysis of variance.

yeast there was significant decrease in the lipid glycerol with food deprivation only in the triglyceride glycerol. With the mixed microbiota of the detrital surfaces and the sand dollars there was an increase in triglyceride glycerol with food deprivation.

Food deprivation causes a relative increase in the phospholipid phosphate of *Neurospora*, yeast and the detrital microbiota (Table II). In the grass shrimp, sand dollars, and amphipods there is a decrease in the phospholipid phosphate with food deprivation.

RATIO OF LIPID GLYCEROL TO LIPID PHOSPHATE

The phospholipids are essential components of membranes and are generally not utilized as endogenous storage materials. In the grass shrimp and sand dollars, the

phospholipid phosphate extracted from fed and food-deprived animals calculated per g dry wt was not significantly different. Loss of phospholipids represents loss of cellular membrane biomass, so the ratio of lipid glycerol to lipid phosphate might be a sensitive measure of nutritional status. Using the ratio of lipid glycerol to lipid phosphate, food

TABLE III

Ratio of glycerol from the total lipids, neutral lipids, and triglyceride to phospholipids in the fungi, estuarine animals and detrital microbiota.

	Total lipid G/LP	Neutral lipid G/LP	Triglyceride G/LP
Neurospora			
Fed	3.3 (0.28)	3.06 (0.27)	1.73 (0.64)
Unfed	0.28 (0.02)*	0.21 (0.06)*	0.16 (0.03)*
Yeast			
Fed	0.34 (0.00)	0.15 (0.02)	0.25 (0.11)
Unfed	0.37 (0.05)	0.19 (0.06)	0.08 (0.02)*
Grass shrimp[a]			
Fed	–	37.0 (2.1)	10.8 (1.1)
Unfed	29.4 (2.1)	12.7 (1.5)*	6.7 (0.6)*
Amphipods[a]			
Fed	174 (24)	105 (38)	88.7 (12)
Unfed	209 (21)	87.2 (20)	87.2 (12)
Sand dollars[b]			
Fed	–	–	86.6 (12)
Unfed	–	–	167 (9.4)*
Detrital microbiota			
Fed	3.64 (0.59)	0.49 (0.14)	0.26 (0.11)
Unfed	1.67 (0.69)*	0.04 (0.02)*	0.09 (0.03)*

[a] Ratio × 1000.
[b] Ratio × 10^6.
* Significant differences (alpha = 0.05) between means.

deprivation induces a significant change in the detrital microbiota, yeast, *Neurospora*, sand dollars, and grass shrimp (Table III).

NUTRITIONAL STATUS OF AMPHIPODS

To check further the effects of laboratory maintenance and ration on the lipid composition, amphipods (chiefly *Melita appendiculata*, *Gammarus mucronatus* and *G. macromucronatus*) were recovered from litter baskets in October 1980 (field samples), held for 7 days in unfiltered sea water in the laboratory on detritus (laboratory) or in the absence of detritus (deprived). The data of Table IV shows that the nutritional status of amphipods held in the laboratory was not significantly different from those recovered from nature and that deprivation from food under these circumstances drastically lowered the triglyceride and ratio of triglyceride to lipid phosphate.

TABLE IV

Comparison of laboratory maintenance for seven days with and without detritus with the field on amphipod triglyceride glycerol and triglyceride glycerol/phospholipid ratio.

	Field	Laboratory	Deprived
Triglyceride glycerol[a]	5.6 (0.49)	4.9 (0.4)	1.02 (0.59)*
Triglyceride glycerol/phospholipid	0.79 (0.08)	0.63 (0.04)	0.13 (0.04)*

[a] $\bar{X}$ (SD), n = 5 replicates, as nmol/animal, 5 animals/sample.
* Significant differences (alpha = 0.05) between means.

DISCUSSION

GLYCEROL ASSAY

Evidence from Table I indicates that the assay of triglyceride as triacetylglycerol is quantitative, reproducible to within a few percent, and sensitive. The glycerol can be quantitatively recovered from the total lipid extract, the neutral lipid fraction, or the isolated triglycerides and is not lost because of volatility if some care is exercised during the steps of the procedure that require removal of water. Since the analysis involves GLC it would be possible to readily determine the rate of triglyceride synthesis or catabolism by measuring the enrichment from ^{13}C labeled precursors. The enrichment is easily determined by operating the GC/MS in the selective ion mode. With precursors of specific activities of over 99% ^{13}C, it is possible to detect enrichments in triglyceride glycerol with exposures to labeled precursors at environmentally relevant concentrations.

FRACTIONATION OF THE LIPIDS

In the fungi and the amphipods, the triglyceride glycerol represented about half the lipid glycerol, while in the detrital microbiota triglyceride glycerol accounted for only 4% of the glycerol in the lipids (Table II). In all the estuarine animals and the fungi examined, it appears that there was little advantage in isolating the triglyceride from the neutral lipid if a relative measure of food deprivation was required. Lee (1974) found in copepods that the ratio of waxes to the phospholipid was a more reliable measure of nutritional status than the total wax ester. In the examination of the mixed microbiota colonizing the Teflon surface exposed in the estuary, the ratio of triglyceride glycerol, neutral lipid glycerol, and total lipid glycerol to phospholipid were all significantly depressed by food deprivation. With the yeast and amphipods, the ratio was not as good an indicator of food deprivation as the glycerol determination.

Sand dollars apparently fulfill Slobodkin's (1962) criterion of an animal whose relative body mass inside the external shell is not critical to survival and the small amount of triglyceride formed does not appear related to the nutritional status. The

molar ratios of triglyceride glycerol to phospholipid differ by a factor of 10^6 rather than 1–100 as in the fungi and animals in which streamlining is more critical to survival.

NUTRITIONAL STUDIES

In many studies it is important to quantitatively document the nutritional status of particular components of the community. In the example used here a 7-day deprivation from food significantly changed the nutritional status measured as the neutral lipid glycerol to phospholipid ratio, whereas allowing the amphipods to graze the detrital microbiota in marine laboratory aquaria did not. These values were compared to organisms recovered from the field.

Since it is now possible to quantitatively describe the detrital microbiota in terms of the biomass, physiological status, metabolic activity and community structure using biochemical measures (Morrison *et al.*, 1977; Bobbie & White, 1980; White *et al.*, 1980a; Bobbie *et al.*, 1981), the effects of amphipod grazing on the microbial activities (Morrison & White, 1980), and on partitioning of the detrital microbial resource between sympatric amphipods (Smith *et al.*, 1982), it is important to document that the grazers themselves are at the same nutritional status in the laboratory as in the field.

Measurements of the nutritional status in prokaryotes by the formation of PHB may prove a very useful technique. Large increases in PHB of the sedimentary prokaryotes occur when the reduced carbon of anaerobic sediments are suddenly aerated (Nickels *et al.*, 1979). Preliminary evidence indicates that rapid PHB synthesis may be a measure of sedimentary bioturbation.

Using a technique that is both sensitive and relatively simple, it is possible to quantitatively compare the nutritional status of a given taxon in the field to defined nutritional states that have been determined by laboratory manipulation for that taxon. In these laboratory studies the nutritional states can be correlated with fecundity, stamina, or a host of other characteristics that would be important in understanding the ecology of this taxon in the field.

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The exposure, recovery and identification of the estuarine detrital animals and microbiota was performed by G. A. Smith; the growth and collection of the fungi by D.J. Uhlinger of this laboratory.

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